

SN. 09/523,809

OGA-10.02 Additional proposed claim amendments

68. (Currently Amended) A method for producing a cultured tissue construct, comprising,
(a) seeding human fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane at about 80% to about 100% confluence, wherein the membrane comprises pores that are about 3 microns or less in size, in a culture vessel in a chemically defined cell culture medium, wherein the chemically defined cell culture medium is free of undefined animal organ or tissue extracts and comprises at least one component selected from the group consisting of: insulin, insulin-like growth factors, transferrin, ascorbate, and ascorbate derivatives;

(b) stimulating the fibroblast cells, which are at least at about 80% confluence to synthesize, secrete and organize extracellular matrix components; and,

(c) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) tenascin; and,
- (iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells on one surface of the porous membrane in the absence of exogenous extracellular matrix components during the culturing conditions, wherein the cultured tissue construct is capable of establishing a graft.

Support for the first amendment can be found on page 18, lines 14-19 of the specification.
Support for the last amendment can be found on page 23, lines 18-19 of the specification.